The Tousled-like kinases regulate genome and epigenome stability: implications in development and disease

[1,2]Sandra Segura-Bayona and [1]Travis H. Stracker

[1]Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Barcelona, Spain

[2]Present address: The Francis Crick Institute, London, UK

[*]To whom correspondence should be addressed. Tel: +34 93 403 11 83; Fax: +34 93 403 71 14; Email: sandra.segura-bayona@crick.ac.uk ORCID (0000-0001-7788-9889) and travis.stracker@irbbarcelona.org ORCID (0000-0002-8650-2081) Institute for Research in Biomedicine (IRB) Barcelona, Department of Oncology, C/ Baldiri Reixac 10, 08028 Barcelona, Spain

Running title: Tousled-like kinases in development and disease

Keywords: kinase, histone chaperone, TLK1, TLK2, ASF1A, ASF1B, DNA replication, DNA repair, genome instability, epigenetics, cancer, cell cycle checkpoint, neurodevelopmental disorder

The Tousled-like kinases regulate genome and epigenome stability: implications in development and disease

Summary

The Tousled-like kinases (TLKs) are an evolutionarily conserved family of serine-threonine kinases that have been implicated in DNA replication, DNA repair, transcription, chromatin structure, viral latency, cell cycle checkpoint control and chromosomal stability in various organisms. The functions of the TLKs appear to depend largely on their ability to regulate the H3/H4 histone chaperone ASF1, although numerous TLK substrates have been proposed. Over the last few years, a clearer picture of TLK function has emerged through the identification of new partners, the definition of specific roles in development and the elucidation of their structural and biochemical properties. In addition, the TLKs have been clearly linked to human disease; both *TLK1* and *TLK2* are frequently amplified in human cancers and *TLK2* mutations have been identified in patients with neurodevelopmental disorders characterized by intellectual disability (ID), autism spectrum disorder (ASD) and microcephaly. A better understanding of the substrates, regulation and diverse roles of the TLKs is needed to understand their functions in neurodevelopment and determine if they are viable targets for cancer therapy. In this review, we will summarize current knowledge of TLK biology and its potential implications in development and disease.

Overview of the Tousled-like kinases

Identification of the Tousled kinase and Tousled-like kinases

The Tousled (TSL) kinase and Tousled-like kinases (TLKs) belong to a distinct branch of nuclear Ser–Thr kinases that are absent in yeast but appear to be constitutively expressed in most cells and tissues from plants and animals. TSL was first identified in *Arabidopsis thaliana* where mutations in the single TSL gene led to pleiotropic defects in morphogenesis, including delays in flowering time and leaf development [1]. Subsequent analysis of TSL showed that its deficiency led to cell cycle abnormalities but its mRNA and protein expression levels were stable throughout the cell cycle [2]. Apart from defects in plant development that could result from proliferation defects [1], *A. thaliana* TSL was reported to directly affect transcriptional gene silencing. Loss of TSL resulted in reduced H3K9me2, associated with heterochromatin, at reactivated gene loci, while no changes were observed in the mitosis-associated phosphorylation of histone H3 on Serine-10 (H3S10) [3]. Additionally, TSL mutants were hypersensitive to UV-B radiation and methyl methanesulfonate (MMS)

and exhibited compromised siRNA-mediated silencing, indicating that TSL loss may lead to transcriptional deregulation and impaired DNA damage repair [4,3].

Following the identification of TSL in *A. thaliana*, Tousled-like kinases (TLKs) were identified in numerous organisms (Figure 1A). This included *Trypanosoma brucei,* which encodes 2 distinct TLKs, as well as*, Drosophila melanogaster* and *Caenorhabditis elegans* that, like *A. thaliana,* encode a single TLK gene. The consequences of TLK depletion have been analyzed during development in each case, further implicating TLK activity in DNA repair, DNA replication, transcription and mitosis [5-8]. At the organismal level, the *TLK-1* gene in *C. elegans* and *Tlk* gene in *D. melanogaster* are essential for viability, as their loss in rapidly dividing cells during early development led to severe chromatin abnormalities, proliferation defects and lethality [5,7]. In *C. elegans*, the major defects identified were transcriptional, reflected by reduced phosphorylation of RNA polymerase II and histone H3 Ser10, a marker of mitosis [5,6]. In *D. melanogaster, Tlk* mutation caused early arrest during embryonic development. This is likely due in part to defective chromatin maintenance, as the deleterious effects in eye development observed following expression of a kinase-dead TLK mutant could be rescued by the overexpression of the histone H3–H4 chaperone ASF1, now the most clearly defined substrate of the TLKs [7,9,10]. Apart from being required for proliferation during development, *Tlk* was also identified in a *D. melanogaster* RNAi screen for cell migration, identifying a requirement for TLK in JAK/STAT activation and the motility of polar cells [11].

Like trypanosomes, mammals encode 2 TLK genes, *TLK1* and *TLK2*, located in different chromosomes (in humans 2q31.1 and 17q23.2, respectively). *TLK1* and *TLK2* share 84% identity at the amino acid (aa) level and 96% identity in the kinase domain (Figure 1B) [12-16]. Each gene is reported to encode several isoforms of unknown relevance and an additional translationally regulated form of TLK1, termed TLK1B, has been characterized [17]. Consistent with data from other organisms, existing evidence suggests important roles for mammalian TLK1 and TLK2 in DNA replication, DNA repair, transcription and organismal development (proposed roles of TLKs in DNA repair were recently reviewed in [18] and will be further summarized here).

Domain organization and structural features of the TLKs

Both TLK1 and TLK2 exhibit the highest levels of activity during S-phase and are regulated by cell cycle checkpoint signaling in response to DNA damage [2,12,9,19,20]. Both TSL and TLKs have a C-terminal protein kinase catalytic domain and a large N-terminal regulatory domain defined by putative coiled coil (CC) domains (Figure 1B) [12,1]. Analysis of the *A. thaliana* TSL protein sequence first revealed the predicted CC regions, as well as three consensus nuclear localization signal (NLS) sequences in the N-terminus and *in vitro* assays showed that the CC regions of the TSL protein were required for oligomerization and full kinase activity [1].

The first X-ray crystal structure of a TLK family kinase domain was recently solved in complex with ATPγS, providing insight into the mode of TLK2 activation and a tool for modeling small molecule inhibitors [21]. *In vitro* analysis of TLK2 activity, as well as the identification of key autophosphorylation sites critical for its activity, indicated that TLK2 is activated through cis-autophosphorylation events in the kinase domain (Figure 1C). These autophosphorylation events trigger a conformational change allowing the trans- and cis-phosphorylation of sites in the N-terminal CC domains and C-tail, similar to what has been described for members of the closely related AGC kinase family, and suggesting that TLKs do not require an activating phosphorylation like members of the CDK family [21,22]. Biochemical studies indicated that monomeric TLK2 cannot achieve full activation and its dimerization and subsequent oligomerization are crucial for maximal activity (Figure 1C). Activated TLK2 dimers led to the appearance of higher order oligomers, which are dependent on autophosphorylation in the loops joining the CC domains. Thus, oligomerization may not only trigger activation but also enzymatic activity by means of recruiting additional TLK2 molecules. It is worth noting that oligomeric constructs are capable of phosphorylating the substrate ASF1a while the kinase domain alone, lacking the N-terminal CC containing segment, cannot [21]. Thus, it is anticipated that either the CC region or its role in dimer/oligomerization are required for substrate recognition.

Most of the autophosphorylation sites identified in TLK2 are found in the loops joining the CC domains, suggesting that they are potentially important regulatory domains *in vivo* [21]. Numerous phosphorylation sites have been identified in the extreme N-terminus of TLK1 and TLK2, which was removed to promote solubility in the structural and biochemical study of the human TLK2 protein [21,23]. This N-terminal region also contains the NLS according to sequence analysis and consistent with the N-terminal mutants lacking the first 160 aa failing

to localize to the nucleus [21,24]. Whether the N-terminal phosphorylation sites represent autophosphorylation or sites of regulation by other kinases remains to be determined. Many additional autophosphorylation sites were identified in the extreme C-terminus of TLK2, downstream of the kinase domain. These included sites analogous to those in TLK1 that were found to be negative regulatory sites targeted for phosphorylation by CHK1 in response to DNA damage [19,20,23].

TLK1 and TLK2 can homo and heterodimerize and this is critically dependent on the first coiled-coil (CC1) domain [21]. Therefore, apart from homodimerization within TLK2 molecules, heterodimerization with TLK1 appears to represent an additional layer of regulation (Figure 1C). Whether the substrate selectivity, activity or regulatory inputs of TLK homodimers and heterodimers differ will be an important question to resolve in future studies.

Interactors and substrates of the Tousled-like kinases

The histone H3/H4 chaperone ASF1 has been identified as an interactor of TLKs in all organisms where it has been examined. In yeast and *C. elegans*, the C-terminus of ASF1 is highly acidic, possibly favoring its interaction with histones (Figure 2). In *D. melanogaster* and mammalian homologs, the C-terminus is instead rich in Ser and Thr residues, which are phosphorylated by the Tousled-like kinases (TLKs) or the DNA-dependent protein kinase (DNA-PKcs) (Figure 2) [9,10,25]. Thus, it is possible that the phosphorylation of the C-terminal tail of human ASF1 might provide the functional equivalent of the acidic C-terminal tail of yeast ASF1.

TLK-dependent phosphorylation sites in mammalian ASF1a and ASF1b have been mapped and functionally investigated (Figure 2). ASF1a is phosphorylated by TLKs during DNA replication on its C-terminal tail residues S166, S175, S192 and S199, while ASF1b is modified on residues S169 and S198 [10]. Although the precise mechanisms by which ASF1 is regulated remain unclear, in *D. melanogaster*, TLK phosphorylation of ASF1 controls its stability, while in vertebrates, TLK1-mediated phosphorylation of several sites on the C-terminal tail of ASF1 promote its binding affinity for the histone H3/H4 heterodimer [10,26]. These data suggested that TLK-mediated phosphorylation of ASF1a and ASF1b may promote histone delivery to downstream histone chaperones, such as CAF1 and HIRA, for replication-coupled and replication-independent chromatin assembly, respectively (Figure 3)

[27]. This is further supported by the observation that the *de novo* deposition of both H3.1 and H3.3 was impaired by TLK depletion [28].

Beyond ASF1, few well-validated substrates of TLKs have been described, although the proposed substrate spectrum of TLK1 consists of more than 150 proteins [29]. TLK1 has been shown to phosphorylate RAD9, a component of the RAD9–RAD1–HUS1 (9-1-1) alternative clamp loader that regulates DNA damage-induced CHK1 activation (Figure 3) [30-35]. Consistent with the TLKs being transiently inhibited by the checkpoint, a DNA damage-induced loss of phosphorylation was observed in RAD9 at S328 [33] and a mild reduction in RAD9 pS328 was reported following Bleomycin treatment of TLK1/2-depleted cells [25]. The interaction between TLK1 and RAD9 and its phosphorylation on T355 was enhanced by DNA damage and implicated in the checkpoint response, and S328 phosphorylation of RAD9 has been shown to regulate its subcellular localization [30,35]. Our own analysis of TLK2 using quantitative IP-mass spectrometry and BioID, as well as other approaches, failed to detect RAD9 as a TLK2 interactor, potentially due to differences in experimental conditions or specificity with TLK1 [36,29]. Future proteomic experiments following acute stress will be needed to fully understand the influence of TLKs on RAD9 and the details of the interaction *in vivo*.

The phosphorylation of H3S10 is required for chromosome condensation and is a widely used marker of mitosis [37]. TLKs have been proposed to mediate H3S10 phosphorylation in various organisms. In human cells, TLK1B phosphorylated H3S10 *in vitro* and TLK1B was capable of complementing a yeast mutant strain lacking the major yeast H3 kinase, Aurora B/IPL1 [13]. In *C. elegans*, TLK-1 promoted the Aurora B-mediated phosphorylation of H3S10 in a kinase independent manner, indicating that the influence on H3S10 is likely an indirect effect (Figure 3) [6]. *Tlk* mutants in *D. melanogaster* displayed reduced levels of phosphorylated histone H3 [7], potentially the result of fewer cells entering mitosis, an effect that has also been observed in human cell lines and trypanosomes [7,28,8]. Therefore, while TLK depletion clearly influences cell cycle progression, it remains unclear whether H3S10 is a direct target of TLK activity *in vivo*.

Using biotinylated TLK1B in a protein array, 164 putative TLK1B interactors, including the NEK1 kinase, which has been implicated in DNA repair, Amyotrophic Lateral Sclerosis (ALS) and ciliogenesis, were identified [29,38,39]. The interaction with NEK1 was enhanced

following DNA damage and TLK1B phosphorylated NEK1 on T141 (Figure 3). Overexpression of a NEK1 T141A mutant influenced cell cycle checkpoint regulation in response to damage[29]. Given that NEK1 activity has been linked to ATR activation, these results may represent further regulatory integration into the checkpoint response [40].

One of the most consistent interactors we and others have identified, aside from ASF1, is LC8-type 1 and 2 (DYNLL1 and DYNLL2) which were originally identified as components of the axonemal dynein motor protein complex [41,36,42,43]. LC8 associates with multiple interaction partners independently of its motor protein functions, including the kinase NEK9 and proteins involved in double-strand break (DSB) repair, such as the MRE11-RAD50-NBS1 complex (MRN), ATMIN/ASCIZ and 53BP1 [44,41,45-47]. LC8 has been proposed to play a general role as a multimerization hub that organizes or stabilizes different protein complexes (Figure 3) [41]. The TLK2 binding domain for LC8 either lies within the CC1 domain or it requires heterodimerization with TLK1, as its binding is lost in CC1 deletion mutants of TLK2 that have impaired TLK1 interactions [21]. However, LC8 does not appear to be a TLK substrate *in vitro*, as purified LC8 is not phosphorylated by active TLK2 [36]. Whether loss of the LC8 interaction impairs other TLK2 interactions or modifies its activity *in vivo* will be interesting to determine, given that phosphorylation of ASF1a by the TLK2-ΔCC1 mutant is reduced *in vitro* and LC8 is implicated in the regulation of the NEK9 kinase that is also regulated via dimerization [21,48].

Another interactor of TLKs identified through unbiased proteomic analysis is the heterochromatin-associated protein RIF1 (Figure 3) [36]. RIF1 was first identified in yeast as a regulator of telomere length [49] while in mammals, RIF1 does not have a telomere specific role but has been implicated in the control of DNA repair and the regulation of replication timing, the latter being a conserved function across species [50-52]. Immunoprecipitation-mass spectrometry (IP-MS) analysis of ASF1a in human MCF-7 cells revealed extensive coverage of both TLK1/2 and RIF1, suggesting the RIF1-TLK interaction may occur through ASF1a [53]. Interestingly, both TLK1 and TLK2, but not ASF1a, were identified in RIF1-associated complexes from IP-MS experiments in mouse ES cells [54]. As RIF1 acts in part through the recruitment of the PP1 phosphatase, it is tempting to speculate that it may act as a direct TLK regulator but further experiments are needed to address this possibility.

Finally, TLKs have been identified as proximal interactors of several key DNA replication and repair factors. While this does not indicate that they are necessarily direct interactors, it provides some insight into the cellular environment of the TLKs and is consistent with the proposition that despite being mostly nuclear soluble proteins they also localize to the vicinity of replication factories [10]. TLK1 and TLK2 were identified in a proteomics screen performed using BioID-PCNA in synchronized S-phase cells, but not in asynchronous cells, regardless of DNA damaging treatments [55]. Moreover, TLK1, TLK2, RIF1 and ASF1a were identified as proximal interactors of 53BP1 using APEX2 labeling, whereas TLK2 was identified as a proximal interactor of the DNA damage response factor MDC1, which also interacts with ASF1a [56,57].

Organismal and cellular functions of TLK activity

Roles of the TLKs in mammalian development

The characterization of TLK1 and TLK2 knockout cells and mice suggested that TLK1 and TLK2 play largely redundant roles in genome maintenance [36] consistent with the fact that they form heterocomplexes [9,58]. Despite the fact that both kinases appear to be largely redundant in homeostatic somatic tissues, *Tlk2*-deficient mice perished during late embryogenesis due to placental failure, while no placental defects were observed in the absence of TLK1 [36]. Reduced ASF1 phosphorylation and impaired expression of placental markers were observed in *Tlk2*-deficient placental tissue. The observation that the knockout of ASF1a in mice leads to lethality by E9.5 [59], notably earlier than that observed with TLK2 deletion, is consistent with the incomplete effect of TLK2 loss on ASF1 phosphorylation we observed [36]. As mRNA levels of *Tlk1* and *Tlk2* in the placenta were similar but relative TLK1 protein levels were strongly reduced in placenta compared with embryonic tissue, it is possible that a translational or post-translational mechanism regulates TLK1 protein levels in this tissue. While several E3 ubiquitin ligases have been identified as TLK1 and TLK2 interactors, additional work is needed to validate these interactions and define their potential roles in post-translational regulation of TLKs in different tissues [60-62].

Bypass of placental development allowed the generation of *Tlk2* null animals that did not show any overt phenotypes in homeostatic conditions, similar to *Tlk1* null mice [36]. While mice appeared anatomically normal, the conditional knockout of *Tlk1* and *Tlk2* in stromal fibroblasts caused increased mammary gland branching and epithelial hyperproliferation

[63]. Available data does not rule out specific functions for TLK1 or TLK2 in the context of particular cell types or in response to stress and suggests that they can largely compensate for each other. Moreover, neither gene acts as a strong tumor suppressor, despite their implication in DNA repair and genome instability. The conditional mouse models will no doubt play an important role in interrogating potential tissue and cell type specific roles of TLK1 and TLK2 in future work.

TLK activity is required for genome and epigenome stability

Human *TLK* expression is constitutive at both the mRNA and protein levels throughout the cell cycle, similar to what had been observed for TSL. However, TLK1/2 protein kinase activity oscillates during the cell cycle, peaking in S-phase [12]. Inhibition of DNA replication with numerous agents inhibited TLK kinase activity in a DNA damage response (DDR)-dependent manner, indicating that TLK activity is linked to ongoing DNA replication and regulated by the checkpoint [12,19,20]. TLK activity has been consistently implicated in the maintenance of genome stability across species but exactly how and why TLK activity is integrated into the DDR and how it promotes genome integrity remains to be fully elucidated.

In yeast, ASF1 interacts directly with and is a substrate of the checkpoint kinase Rad53 and this interaction has been implicated in genome stability and cell cycle checkpoint recovery [64,65]. In human cells, ASF1 does not appear to interact directly with the checkpoint kinases, but is instead regulated by them indirectly through the TLKs. CHK1 phosphorylates TLK1 at the C-terminal S695 residue, reversibly inhibiting its activity [19,20]. This CHK1-dependent modulation of TLK1 potentially coordinates global ASF1 histone-binding capacity with the checkpoint response and allows chromatin restructuring during DNA repair. The attenuation of TLK1 activity upon checkpoint activation is transient and TLK1 was identified again as a direct CHK1 target using an analog sensitive CHK1 allele, although the physical interaction of CHK1 with TLK1B or TLK2 has not been observed in proteomics studies [36,29,66]. This may reflect the fact that most studies have been performed in asynchronous cells in the absence of DNA damage or that the interaction is too transient to be detected by the methods used. Although similar phosphorylated sites exist in the C-terminus of TLK2, it remains unclear if TLK2 is directly regulated by CHK1 or through heterodimerization with C-terminally phosphorylated TLK1.

Multiple lines of evidence have implicated TLK activity in the control of cell cycle progression, both in asynchronous cells and cells with DNA damage. The previously described interaction of TLK1 with RAD9, that has multiple roles in the response to DNA damage, has been linked to G2/M checkpoint recovery [30,32,33], although other reports implicated TLK2, but not TLK1, in G2/M checkpoint recovery through ASF1a-mediated transcriptional regulation [67] (Figure 3). Conversely, TLK2 overexpression was also shown to impair the DNA damage-induced G2/M checkpoint in human cancer cells and *Tlk* overexpression prolonged G2 in *D. melanogaster* independently of its activity [68,69]. These may explain the observation that overexpression of TLK1B in mouse cells confers enhanced resistance to ionizing radiation, that is more toxic in highly proliferative cells [13]. Thus, the regulation of TLK levels and activity is required for normal cell cycle progression, likely involving numerous interactions and kinase-dependent and independent functions.

In addition to cell cycle progression, TLK activity has been implicated in chromosome segregation. The overexpression of a dominant negative form of TLK1B caused chromosome missegregation in mouse cells [70] and TLK1 was proposed to regulate myosin II regulatory light chain (MRLC) during mitosis to maintain correct chromosome segregation [71]. Further, mitotic defects have been observed in worms, flies and trypanosomes, although whether these are the result of under-replicated DNA, cell cycle progression defects or bona fide mitotic roles of TLK activity remains to be determined [8,7,6].

In cancer cells, depletion of TLK activity impaired nucleosome assembly and led to replication-coupled ssDNA accumulation and fork stalling [28], a state known as replication stress [72]. DSBs accumulated over time, inducing the DDR and provoking p53 activation and G1 arrest. TLK-depleted cells were sensitized to treatment with checkpoint kinase or Poly(ADP-ribose) Polymerase (PARP) inhibitors, indicating that ATR/CHK1 and PARP activity were crucial to prevent the rapid collapse of forks arrested due to chromatin assembly defects [28]. New histones incorporated during DNA replication can be identified by the lack of H4K20 methylation (H4K20me0) that acts to signal the recruitment of the TONSL-MMS22L homologous recombination complex [73]. Long term (72 hours) depletion of CAF1 and ASF1 impaired the recruitment of TONSL-MMS22L to DNA double-strand breaks [25]. Impaired nucleosome assembly in TLK-deficient cells would be predicted to have a similar effect on TONSL-MMS22L recruitment that could sensitize replication forks

to collapse due to ATR/CHK1-dependent suppression of new origin firing and RPA exhaustion [74].

In addition to its well-established roles in transcription and replication, ASF1a was recently shown to regulate DSB repair. ASF1a is phosphorylated in a DNA damage-dependent manner by DNA-PKcs at S192, a residue previously identified to be a TLK target, indicating that multiple signaling pathways converge on the C-terminal tail of ASF1 (Figure 2) [10,25]. This phosphorylation event promotes MMS22L-TONSL chromatin loading and subsequent recruitment of the RAD51 recombinase to promote homologous recombination (HR)-mediated DNA repair. Paradoxically, ASF1a has also been proposed to suppress HR and promote non-homologous end-joining (NHEJ), a competing DSB repair pathway, through its ability to interact with MDC1 and promote the recruitment of several key factors, including the ubiquitin ligases RNF8 and RNF168 [57]. Considering that DNA damage-induced checkpoint activation transiently inhibits TLK activity [19] and ASF1a pS192 occurs after DNA damage, even in TLK1/2-depleted cells [25], it seems unlikely that TLK activity plays a major regulatory role. Nevertheless, as TLK activity plays an important role in genome stability and interacts with RIF1, a key regulator of DNA repair pathway choice, this possibility warrants further investigation [75].

Despite available evidence indicating that ASF1 is the primary TLK target in metazoans [9], it is notable that TLK depletion does not simply phenocopy ASF1 loss. Depletion of total ASF1 reduced replication fork speed and caused a strong S-phase arrest without causing RPA accumulation or DDR checkpoint activation [28,76]. Treatment with the deoxyribonucleotide reductase (RNR) inhibitor hydroxyurea (HU), that generates a robust DDR in cycling cells, including ssDNA/RPA accumulation and DNA breaks, failed to do so in ASF1-depleted cells [76]. These data, as well as the fact that the inhibition of DNA replication in TLK-depleted cells ameliorated the levels or replication stress and DNA damage, indicated that ongoing DNA replication underlies much of the genomic instability that accumulates in TLK-depleted cells, consistent with its peak activity in S-phase [28]. This may reflect that ASF1 has additional functions that are independent of TLK regulation, such as its interactions with the MCM2-7 helicase and role in histone recycling, and/or that additional TLK substrates influence the phenotypes [76,77].

In addition to promoting genome stability, several lines of evidence indicate that TLKs play an important role in epigenome maintenance [5,3,67]. TLK2 was identified in an siRNA screen for proteins required to maintain the silencing of Kaposi's sarcoma-associated herpesvirus (KSHV) and TLK1 depletion also resulted in reactivation of Epstein-Barr virus (EBV) [78]. In addition to exogenous viruses, the impaired *de novo* nucleosome deposition we observed in TLK-depleted cells [28] would be predicted to have potential consequences for epigenome maintenance that is required for cell identity programs, as well as the silencing of non-coding regions, such as endogenous viruses and telomeres, where ASF1 has been previously implicated [79,80]. Notably, we observed a strong decrease in H3.3 deposition in TLK-depleted cells and this replacement variant of H3 plays a key role in heterochromatin formation at telomeres and other transcriptionally silent genomic regions, as well as in promoters of developmentally regulated genes [81,82,28,83,84].

Roles of TLK activity in human disease

Despite their implication in replication stress, genome and epigenome instability and hyperproliferation, all of which play key roles in cancer etiology, both *TLK1* and *TLK2* are often maintained or amplified in human cancers and few recurrent mutations or copy number losses have been identified [28,85,86,63]. This pattern is reminiscent to that of ATR, and to a lesser extent CHK1, that is required by many cancers to tolerate increased replication stress [87]. These and other data have suggested that TLK activity may be a promising target to explore in cancer treatment. In addition, recent genetic studies have now implicated *TLK2* mutations in several neurodevelopmental disorders, including intellectual disability (ID) and autism spectrum disorder (ASD), often associated with microcephaly [88-90], raising new interest in understanding the precise developmental roles of TLK activity.

TLK activity as a therapeutic target in cancer

In breast cancer, amplification of the 17q23 region, that contains several candidate oncogenes, including *TLK2*, occurs in more than 40% of tumors. In addition, several *TLK2* single nucleotide polymorphisms (SNP) of unknown function, including rs733025 and rs2245092, were significantly associated with breast cancer risk and hormone receptor–positive breast tumors [91,92]. *TLK2* was amplified in luminal ER+ breast cancer and was found to be hyper-phosphorylated in proteogenomics studies, potentially indicating increased activity [93,21,28,94]. *TLK2* overexpression also correlated with increased chromosomal instability (CIN) in breast cancer [68] and promoted cell invasion and migration, both

characteristics associated with metastasis in luminal breast cancer cells [93]. Moreover, a therapeutic effect of TLK2 inhibition or depletion was observed in xenograft models of breast cancer and glioblastoma [93,63].

Analysis of *TLK1/2* copy number alterations across pan-cancer genomes showed that TLKs are more frequently maintained or amplified than lost [28]. In addition, high *TLK1* and *TLK2* expression levels correlated with poor prognosis in several cancer cohorts, including cervical squamous cell carcinoma and endocervical adenocarcinoma (TCGA-cesc) and uveal melanoma (TCGA-uvm). Targeting TLK activity was proposed as a potential therapeutic intervention in prostate cancer and shown to enhance the effects of some chemotherapeutic agents, including ATR/CHK1 and PARP inhibitors, as well as cisplatin, in different cancer types [28,95-97]. How *TLK1* or *TLK2* expression is misregulated in cancer remains largely unclear. Aside from copy number alterations, miR-16 was shown to regulate TLK1 levels in oral squamous cell carcinoma and the circadian E3 ligase complex was demonstrated to regulate TLK2 stability, suggesting that alterations in these mechanisms could influence TLK levels in some types of cancer [98,62].

In addition to playing direct roles in DNA replication and chromatin maintenance, TLK1 and TLK2 were identified as non-cell autonomous modifiers of RAS pathway signaling in worms and mice [63]. Conditional depletion of TLK1 or TLK2 in mouse mammary fibroblasts caused hyperproliferation of surrounding mammary epithelial cells, indicating that loss of TLK activity leads to cellular crosstalk that may be relevant to their role in cancer and as therapeutic targets. Further supporting an important role for TLK depletion provoking a secretory response, depletion of Tlk in flies influenced cytokine-dependent signaling during cell migration and our recent work has demonstrated that TLK depletion leads to the loss of heterochromatin maintenance, desilencing of repetitive elements, including ERVs, and the activation of an innate immune response that included the secretion of inflammatory cytokines [84,11].

The emerging picture suggests that like ATR-CHK1, some degree of TLK activity is required for cancer cell proliferation and preventing the accumulation of toxic levels of replication stress. This requirement may be elevated in highly proliferative cancer cells and demand the amplification of the TLK-ASF1 pathway to avoid replication stress. Therefore, targeting TLK activity could have a therapeutic benefit in cancer and additionally, it could potentially

augment novel selective therapies, including cell cycle checkpoint inhibitors, PARP inhibitors and DNA damaging agents [28]. Several small molecule TLK inhibitors have been reported, although most of these are highly promiscuous or target many non-kinase proteins, thus potent specific inhibitors have yet to be identified [21,93,99]. Future work exploiting the TLK2 crystal structure as a tool for rational inhibitor design could conceivably identify clinically effective agents for use in cancer treatment [21].

TLK2 mutations in neurodevelopmental disorders

Neurodevelopmental disorders, including ASD and ID, are commonly caused by *de novo* spontaneous or inherited genetic mutations that affect brain development. A meta-analysis of data from over two thousand patients identified *TLK2* as one of ten new candidate genes for ID and other neurodevelopmental disorders, such as ASD and schizophrenia [88]. These patients have *de novo* loss of function mutations (DNM) and exhibit severe clinical features, such as facial dysmorphisms and microcephaly. Previous studies had identified DNMs in *TLK2* in sporadic ASD [100] and schizophrenia [101]. The fact that *TLK2* DNM are significantly enriched in ASD was also confirmed by an independent study of a Japanese cohort [90].

A subsequent study involving patients from up to 7 countries characterized 38 unrelated individuals with two affected mothers with heterozygous variants in the *TLK2* gene with a distinct neurodevelopmental disorder with a consistent pathological spectrum, including mild developmental delay, behavioral disorders, gastro-intestinal problems and facial dysmorphisms [89]. Mutations in the *TLK2* gene include loss-of-function (LOF) variants (4 frameshift, 10 nonsense variants and one balanced translocation resulting in a disruption between *TLK2* exon 2 and 3) and missense variants (9 missense and 12 canonical splice-site variants) (Figure 4A). While *TLK1* mutations have not been statistically linked to any distinct neurodevelopmental disorder, DNMs have been reported in isolated cases of neurodevelopmental disorder patients, suggesting that its role in brain development warrants further investigation [102,103,88,104]. It is worth noting that both *TLK1* and *TLK2* (MIM number 608438 and 608439 respectively) are significantly intolerant for both missense and truncating mutations in healthy individuals, similar to what has been observed in cancer genomes [89,28].

TLK2 mutations were found in heterozygosity, indicating that the neurodevelopmental defects presumably arise due to haploinsufficiency. We have previously speculated that the effects of the ID/ASD related *TLK2* mutations could reflect placental defects, although we have not observed any clear placental phenotypes in *Tlk2* heterozygous mice and the possibility that *TLK1* mutations may underlie similar disorders would argue against this as being the sole cause [36,89]. Four previously described *TLK2* ID mutations [88] strongly reduced kinase activity *in vitro* [21], suggesting that at least some of the *TLK2* missense mutations could have a mild dominant negative effect. However, in other cases that involve larger truncations that include the CC1 domain involved in dimerization, haploinsufficiency more likely accounts for the related pathologies.

The neural progenitor population is particularly sensitive to cell cycle delays and DNA damage. Attrition of these cells is one of the major underlying causes of several neurodevelopmental disorders, including Seckel Syndrome, which is characterized by short stature and microcephaly [105], both of which are observed in a number of patients with heterozygous *TLK2* mutations [89]. Hypomorphic mutations in both *ATR* and several *MCM* components of the replicative helicase have been linked to replication stress and placental defects, in the latter case associated with inflammatory responses [106,107]. However, analysis of *Tlk2*-deficient murine placentas did not uncover increased DNA damage signaling or proliferative defects, although this cannot be ruled out in humans due to major differences in placental development and gestation time [36].

Alternatively, defects in H3.3 deposition could compromise epigenetic maintenance [28]. H3.3 is required for H3K9me3 establishment in telomeres and endogenous retroviral elements (ERVs), as well as for H3K27me3 establishment at promoters of developmentally regulated genes [81-83]. Consistent with this, depletion of TLK1 delayed downregulation of pluripotency genes and impaired embryonic stem cell differentiation, suggesting impaired histone-mediated regulation of differentiation programs [108]. Numerous mutations in genes involved in epigenetic maintenance have been identified in ASD, including *ATRX,* that plays a prominent role in H3.3 deposition in heterochromatin at retrotransposons and telomeres [109,81], as well as *KDM5B, KDM5C, SETD5*, and *DNMT3A* [110]. Treatment with the histone deacetylase inhibitor valproic acid (VPA) led to increased ERV expression, and prenatal exposure to VPA has been linked to autism, identifying links between chromatin silencing, placental formation and autism [110-112].

An additional, non-exclusive possibility is that TLK activity regulates microexon splicing. Brain-specific microexon splicing defects have been identified as a possible molecular mechanism underlying idiopathic ASD, given that a significant fraction of autistic brains analyzed by transcriptomic profiling showed misregulation of microexons and reduced levels of regulators of neuronal alternative splicing [113,114]. A recent CRISPR-screen identified around 200 regulators of neuronal microexon splicing, several of which are often disrupted in ASD [115]. TLK2 was identified as a positive regulator of microexon splicing and was implicated in the stability of the alternative splicing factor SRRM4, which is required for microexon inclusion [115]. In addition to TLK2, the H3.3 chaperone HIRA was also identified, suggesting that *TLK2* mutations could potentially impact neuronal microexon splicing through its role in regulating ASF1a and H3.3 deposition [28]. Although a mechanistic link between histone variant usage and microexon splicing has not been reported to our knowledge, post-translational modifications of H3.3 and its influence on the transcriptional elongation rate have both been implicated in splicing defects [116,117].

In future work, it will therefore be interesting to determine whether *TLK2* mutations in human patients compromise ERV silencing, impair DNA replication, affect microexon splicing or elicit inflammatory responses that have been associated with ASD and other neurodevelopmental disorders, as well as placental defects in animal models (Figure 4B) [118-120,107,121,122,84].

Conclusions and open questions

Despite the recent advances in our understanding of TLK structure, function and roles in human disease, many open questions remain about its regulation, targets and cell type specific roles. While TLK activity is clearly important for genomic stability and regulated by the cell cycle checkpoint machinery, why is it important to rapidly inhibit TLK following DNA damage? How does reduced TLK activity cause replication stress and how is ssDNA generated at forks stalled by reduced TLK activity? Are other TLK substrates aside from ASF1 relevant to fork progression? Is TLK activity important in post-mitotic cell populations? Structurally, we now have a clearer picture of the kinase domain but many questions remain. What is the structure of the CC domains and their polarity in the context of TLK dimers or multimers? How do they influence subcellular localization, activity or substrate selection? How does the phosphorylation of the C-terminus of TLK1 by CHK1

influence activity and does this apply to TLK2? Finally, as it is clear that TLKs are important during development and even appear to be selected for in cancer, a detailed analysis of their cell type and tissue specific roles will be needed. Is the influence of TLK mutations on neurodevelopment cell autonomous and if so, what cell types are affected and how? Would targeting TLK activity in cancer represent a viable strategy? Addressing these and other questions will further our insight into the important roles of these poorly-understood kinases in genome and epigenome maintenance.

Acknowledgements

We are grateful to members of the Stracker lab and A. Groth for discussions of unpublished data and suggestions. We apologize to those colleagues whose relevant work could not be specifically mentioned due to space constraints.

Funding

THS was funded by the Spanish Ministry of Science, Innovation and Universities (BFU2015-68354/GENPATH, GINDATA and FEDER, the Centres of Excellence Severo Ochoa award and the CERCA Programme. SSB was funded by a PhD fellowship and the project LCF/PR/GN14/10270002 from the "la Caixa" Foundation.

Competing interests

The authors declare no competing interests.

Figure Legends

Figure 1. A. Phylogenetic tree generated by Clustal Omega and domain organization of TSL/TLKs across organisms. The total number of residues of each protein is shown. B. Domain architecture of human TLKs. C. Schematic of activation mechanism of human TLKs based on structural and biochemical studies [21].

Figure 2. Comparison of yeast ASF1, the 2 *C. elegans* ASF1 proteins (encoded by the *unc-85* and *asfl-1* genes) and human ASF1a and ASF1b proteins. The green boxes show the conserved histone chaperone domain of about 70% similarity between human and budding

yeast/*C. elegans*. The red boxes show the acidic region present in both yeast and *C. elegans* ASF1. The phosphosites identified in the C-ter tail of human ASF1a/b are indicated [10,25,26]. The total number of residues of each protein is shown. In the right panel, sequence identity and similarity between the 1-156 aa of yeast (y), C. elegans (ce) and human (h) ASF1 are displayed as a percentage and were assessed by NCBI Blast (blastp suite-2sequences).

Figure 3. Schematic summary model of the proposed functional roles of TLKs through various interactors and substrates involved in genome and epigenome stability. See main text for details.

Figure 4. A. Diagram of the TLK2 protein (Q86UE8) mapping all of the human *TLK2* mutations identified in patients with neurodevelopmental disorders, ID or ASD [89,88]. For splicing variants, the annotated transcript variant corresponding to Q86UE8-1 (NM_001284333.1) was used. B. Summary schematic of the possible underlying mechanisms for *TLK2* mutations in human neurodevelopmental disorders. See main text for details.

References

1. Roe JL, Rivin CJ, Sessions RA, Feldmann KA, Zambryski PC (1993) The Tousled gene in A. thaliana encodes a protein kinase homolog that is required for leaf and flower development. Cell 75 (5):939-950. doi:0092-8674(93)90537-Z [pii]
2. Ehsan H, Reichheld JP, Durfee T, Roe JL (2004) TOUSLED kinase activity oscillates during the cell cycle and interacts with chromatin regulators. Plant physiology 134 (4):1488-1499. doi:10.1104/pp.103.038117
3. Wang Y, Liu J, Xia R, Wang J, Shen J, Cao R, Hong X, Zhu JK, Gong Z (2007) The protein kinase TOUSLED is required for maintenance of transcriptional gene silencing in Arabidopsis. EMBO Rep 8 (1):77-83. doi:10.1038/sj.embor.7400852
4. Uddin MN, Dunoyer P, Schott G, Akhter S, Shi C, Lucas WJ, Voinnet O, Kim JY (2014) The protein kinase TOUSLED facilitates RNAi in Arabidopsis. Nucleic Acids Res 42 (12):7971-7980. doi:10.1093/nar/gku422
5. Han Z, Saam JR, Adams HP, Mango SE, Schumacher JM (2003) The C. elegans Tousled-like kinase (TLK-1) has an essential role in transcription. Curr Biol 13 (22):1921-1929. doi:S0960982203008030 [pii]
6. Han Z, Riefler GM, Saam JR, Mango SE, Schumacher JM (2005) The C. elegans Tousled-like kinase contributes to chromosome segregation as a substrate and regulator of the Aurora B kinase. Curr Biol 15 (10):894-904. doi:S0960-9822(05)00394-5 [pii]
10.1016/j.cub.2005.04.019
7. Carrera P, Moshkin YM, Gronke S, Sillje HH, Nigg EA, Jackle H, Karch F (2003) Tousled-like kinase functions with the chromatin assembly pathway regulating nuclear divisions. Genes Dev 17 (20):2578-2590. doi:10.1101/gad.276703
17/20/2578 [pii]
8. Li Z, Gourguechon S, Wang CC (2007) Tousled-like kinase in a microbial eukaryote regulates spindle assembly and S-phase progression by interacting with Aurora kinase and chromatin assembly factors. J Cell Sci 120 (Pt 21):3883-3894. doi:jcs.007955 [pii]
10.1242/jcs.007955
9. Sillje HH, Nigg EA (2001) Identification of human Asf1 chromatin assembly factors as substrates of Tousled-like kinases. Curr Biol 11 (13):1068-1073. doi:S0960-9822(01)00298-6 [pii]
10. Klimovskaia IM, Young C, Stromme CB, Menard P, Jasencakova Z, Mejlvang J, Ask K, Ploug M, Nielsen ML, Jensen ON, Groth A (2014) Tousled-like kinases phosphorylate Asf1 to promote histone supply during DNA replication. Nature communications 5:3394. doi:10.1038/ncomms4394
11. Xiang W, Zhang D, Montell DJ (2016) Tousled-like kinase regulates cytokine-mediated communication between cooperating cell types during collective border cell migration. Mol Biol Cell 27 (1):12-19. doi:10.1091/mbc.E15-05-0327
12. Sillje HH, Takahashi K, Tanaka K, Van Houwe G, Nigg EA (1999) Mammalian homologues of the plant Tousled gene code for cell-cycle-regulated kinases with maximal activities linked to ongoing DNA replication. EMBO J 18 (20):5691-5702. doi:10.1093/emboj/18.20.5691
13. Li Y, DeFatta R, Anthony C, Sunavala G, De Benedetti A (2001) A translationally regulated Tousled kinase phosphorylates histone H3 and confers radioresistance when overexpressed. Oncogene 20 (6):726-738. doi:10.1038/sj.onc.1204147
14. Shalom S, Don J (1999) Tlk, a novel evolutionarily conserved murine serine threonine kinase, encodes multiple testis transcripts. Mol Reprod Dev 52 (4):392-405. doi:10.1002/(SICI)1098-2795(199904)52:4<392::AID-MRD8>3.0.CO;2-Y
15. Yamakawa A, Kameoka Y, Hashimoto K, Yoshitake Y, Nishikawa K, Tanihara K, Date T (1997) cDNA cloning and chromosomal mapping of genes encoding novel protein kinases

termed PKU-alpha and PKU-beta, which have nuclear localization signal. Gene 202 (1-2):193-201
16. Zhang S, Xing H, Muslin AJ (1999) Nuclear localization of protein kinase U-alpha is regulated by 14-3-3. J Biol Chem 274 (35):24865-24872
17. Sunavala-Dossabhoy G, Fowler M, De Benedetti A (2004) Translation of the radioresistance kinase TLK1B is induced by gamma-irradiation through activation of mTOR and phosphorylation of 4E-BP1. BMC Mol Biol 5:1. doi:10.1186/1471-2199-5-1
1471-2199-5-1 [pii]
18. Sunavala-Dossabhoy G (2018) Preserving salivary gland physiology against genotoxic damage - the Tousled way. Oral Dis 24 (8):1390-1398. doi:10.1111/odi.12836
19. Groth A, Lukas J, Nigg EA, Sillje HH, Wernstedt C, Bartek J, Hansen K (2003) Human Tousled like kinases are targeted by an ATM- and Chk1-dependent DNA damage checkpoint. EMBO J 22 (7):1676-1687. doi:10.1093/emboj/cdg151
20. Krause DR, Jonnalagadda JC, Gatei MH, Sillje HH, Zhou BB, Nigg EA, Khanna K (2003) Suppression of Tousled-like kinase activity after DNA damage or replication block requires ATM, NBS1 and Chk1. Oncogene 22 (38):5927-5937. doi:10.1038/sj.onc.1206691
1206691 [pii]
21. Mortuza GB, Hermida D, Pedersen AK, Segura-Bayona S, López-Méndez B, Redondo P, Garrote AM, Muñoz IG, Villamor-Paya M, Jauset C, Olsen JV, Stracker TH, Montoya G (2018) Molecular basis of Tousled Like Kinase 2 activation. Nature communications 9 (1):2535. doi:10.1038/s41467-018-04941-y
22. Leroux AE, Schulze JO, Biondi RM (2018) AGC kinases, mechanisms of regulation and innovative drug development. Semin Cancer Biol 48:1-17. doi:10.1016/j.semcancer.2017.05.011
23. Hornbeck PV, Zhang B, Murray B, Kornhauser JM, Latham V, Skrzypek E (2015) PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. Nucleic Acids Res 43 (Database issue):D512-520. doi:10.1093/nar/gku1267
24. Kosugi S, Hasebe M, Tomita M, Yanagawa H (2009) Systematic identification of cell cycle-dependent yeast nucleocytoplasmic shuttling proteins by prediction of composite motifs. Proc Natl Acad Sci U S A 106 (25):10171-10176. doi:10.1073/pnas.0900604106
25. Huang TH, Fowler F, Chen CC, Shen ZJ, Sleckman B, Tyler JK (2018) The Histone Chaperones ASF1 and CAF-1 Promote MMS22L-TONSL-Mediated Rad51 Loading onto ssDNA during Homologous Recombination in Human Cells. Mol Cell 69 (5):879-892 e875. doi:10.1016/j.molcel.2018.01.031
26. Pilyugin M, Demmers J, Verrijzer CP, Karch F, Moshkin YM (2009) Phosphorylation-mediated control of histone chaperone ASF1 levels by Tousled-like kinases. PLoS One 4 (12):e8328. doi:10.1371/journal.pone.0008328
27. Hammond CM, Stromme CB, Huang H, Patel DJ, Groth A (2017) Histone chaperone networks shaping chromatin function. Nat Rev Mol Cell Biol 18 (3):141-158. doi:10.1038/nrm.2016.159
28. Lee SB, Segura-Bayona S, Villamor-Paya M, Saredi G, Todd MAM, Attolini CS, Chang TY, Stracker TH, Groth A (2018) Tousled-like kinases stabilize replication forks and show synthetic lethality with checkpoint and PARP inhibitors. Sci Adv 4 (8):eaat4985. doi:10.1126/sciadv.aat4985
29. Singh V, Connelly ZM, Shen X, De Benedetti A (2017) Identification of the proteome complement of humanTLK1 reveals it binds and phosphorylates NEK1 regulating its activity. Cell Cycle:0. doi:10.1080/15384101.2017.1314421
30. Kelly R, Davey SK (2013) Tousled-like kinase-dependent phosphorylation of Rad9 plays a role in cell cycle progression and G2/M checkpoint exit. PLoS One 8 (12):e85859. doi:10.1371/journal.pone.0085859

31. Delacroix S, Wagner JM, Kobayashi M, Yamamoto K, Karnitz LM (2007) The Rad9-Hus1-Rad1 (9-1-1) clamp activates checkpoint signaling via TopBP1. Genes Dev 21 (12):1472-1477. doi:10.1101/gad.1547007
32. Canfield C, Rains J, De Benedetti A (2009) TLK1B promotes repair of DSBs via its interaction with Rad9 and Asf1. BMC Mol Biol 10:110. doi:1471-2199-10-110 [pii] 10.1186/1471-2199-10-110
33. Sunavala-Dossabhoy G, De Benedetti A (2009) Tousled homolog, TLK1, binds and phosphorylates Rad9; TLK1 acts as a molecular chaperone in DNA repair. DNA Repair (Amst) 8 (1):87-102. doi:S1568-7864(08)00354-6 [pii] 10.1016/j.dnarep.2008.09.005
34. Lieberman HB (2006) Rad9, an evolutionarily conserved gene with multiple functions for preserving genomic integrity. J Cell Biochem 97 (4):690-697. doi:10.1002/jcb.20759
35. Awate S, De Benedetti A (2016) TLK1B mediated phosphorylation of Rad9 regulates its nuclear/cytoplasmic localization and cell cycle checkpoint. BMC Mol Biol 17:3. doi:10.1186/s12867-016-0056-x
36. Segura-Bayona S, Knobel PA, Gonzalez-Buron H, Youssef SA, Pena-Blanco A, Coyaud E, Lopez-Rovira T, Rein K, Palenzuela L, Colombelli J, Forrow S, Raught B, Groth A, de Bruin A, Stracker TH (2017) Differential requirements for Tousled-like kinases 1 and 2 in mammalian development. Cell Death Differ. doi:10.1038/cdd.2017.108
37. Crosio C, Fimia GM, Loury R, Kimura M, Okano Y, Zhou H, Sen S, Allis CD, Sassone-Corsi P (2002) Mitotic phosphorylation of histone H3: spatio-temporal regulation by mammalian Aurora kinases. Mol Cell Biol 22 (3):874-885
38. Fry AM, Bayliss R, Roig J (2017) Mitotic Regulation by NEK Kinase Networks. Front Cell Dev Biol 5:102. doi:10.3389/fcell.2017.00102
39. Kenna KP, van Doormaal PT, Dekker AM, Ticozzi N, Kenna BJ, Diekstra FP, van Rheenen W, van Eijk KR, Jones AR, Keagle P, Shatunov A, Sproviero W, Smith BN, van Es MA, Topp SD, Kenna A, Miller JW, Fallini C, Tiloca C, McLaughlin RL, Vance C, Troakes C, Colombrita C, Mora G, Calvo A, Verde F, Al-Sarraj S, King A, Calini D, de Belleroche J, Baas F, van der Kooi AJ, de Visser M, Ten Asbroek AL, Sapp PC, McKenna-Yasek D, Polak M, Asress S, Munoz-Blanco JL, Strom TM, Meitinger T, Morrison KE, Consortium S, Lauria G, Williams KL, Leigh PN, Nicholson GA, Blair IP, Leblond CS, Dion PA, Rouleau GA, Pall H, Shaw PJ, Turner MR, Talbot K, Taroni F, Boylan KB, Van Blitterswijk M, Rademakers R, Esteban-Perez J, Garcia-Redondo A, Van Damme P, Robberecht W, Chio A, Gellera C, Drepper C, Sendtner M, Ratti A, Glass JD, Mora JS, Basak NA, Hardiman O, Ludolph AC, Andersen PM, Weishaupt JH, Brown RH, Jr., Al-Chalabi A, Silani V, Shaw CE, van den Berg LH, Veldink JH, Landers JE (2016) NEK1 variants confer susceptibility to amyotrophic lateral sclerosis. Nat Genet 48 (9):1037-1042. doi:10.1038/ng.3626
40. Liu S, Ho CK, Ouyang J, Zou L (2013) Nek1 kinase associates with ATR-ATRIP and primes ATR for efficient DNA damage signaling. Proc Natl Acad Sci U S A 110 (6):2175-2180. doi:10.1073/pnas.1217781110
41. Rapali P, Szenes A, Radnai L, Bakos A, Pal G, Nyitray L (2011) DYNLL/LC8: a light chain subunit of the dynein motor complex and beyond. FEBS J 278 (17):2980-2996. doi:10.1111/j.1742-4658.2011.08254.x
42. Hutchins JR, Toyoda Y, Hegemann B, Poser I, Heriche JK, Sykora MM, Augsburg M, Hudecz O, Buschhorn BA, Bulkescher J, Conrad C, Comartin D, Schleiffer A, Sarov M, Pozniakovsky A, Slabicki MM, Schloissnig S, Steinmacher I, Leuschner M, Ssykor A, Lawo S, Pelletier L, Stark H, Nasmyth K, Ellenberg J, Durbin R, Buchholz F, Mechtler K, Hyman AA, Peters JM (2010) Systematic analysis of human protein complexes identifies chromosome segregation proteins. Science 328 (5978):593-599. doi:10.1126/science.1181348

43. Hein MY, Hubner NC, Poser I, Cox J, Nagaraj N, Toyoda Y, Gak IA, Weisswange I, Mansfeld J, Buchholz F, Hyman AA, Mann M (2015) A human interactome in three quantitative dimensions organized by stoichiometries and abundances. Cell 163 (3):712-723. doi:10.1016/j.cell.2015.09.053
44. Rapali P, Garcia-Mayoral MF, Martinez-Moreno M, Tarnok K, Schlett K, Albar JP, Bruix M, Nyitray L, Rodriguez-Crespo I (2011) LC8 dynein light chain (DYNLL1) binds to the C-terminal domain of ATM-interacting protein (ATMIN/ASCIZ) and regulates its subcellular localization. Biochem Biophys Res Commun 414 (3):493-498. doi:10.1016/j.bbrc.2011.09.093
45. Lo KW, Kan HM, Chan LN, Xu WG, Wang KP, Wu Z, Sheng M, Zhang M (2005) The 8-kDa dynein light chain binds to p53-binding protein 1 and mediates DNA damage-induced p53 nuclear accumulation. J Biol Chem 280 (9):8172-8179. doi:10.1074/jbc.M411408200
46. He YJ, Meghani K, Caron MC, Yang C, Ronato DA, Bian J, Sharma A, Moore J, Niraj J, Detappe A, Doench JG, Legube G, Root DE, D'Andrea AD, Drane P, De S, Konstantinopoulos PA, Masson JY, Chowdhury D (2018) DYNLL1 binds to MRE11 to limit DNA end resection in BRCA1-deficient cells. Nature 563 (7732):522-526. doi:10.1038/s41586-018-0670-5
47. Becker JR, Cuella-Martin R, Barazas M, Liu R, Oliveira C, Oliver AW, Bilham K, Holt AB, Blackford AN, Heierhorst J, Jonkers J, Rottenberg S, Chapman JR (2018) The ASCIZ-DYNLL1 axis promotes 53BP1-dependent non-homologous end joining and PARP inhibitor sensitivity. Nature communications 9 (1):5406. doi:10.1038/s41467-018-07855-x
48. Regue L, Sdelci S, Bertran MT, Caelles C, Reverter D, Roig J (2011) DYNLL/LC8 protein controls signal transduction through the Nek9/Nek6 signaling module by regulating Nek6 binding to Nek9. J Biol Chem 286 (20):18118-18129. doi:10.1074/jbc.M110.209080
49. Shi T, Bunker RD, Mattarocci S, Ribeyre C, Faty M, Gut H, Scrima A, Rass U, Rubin SM, Shore D, Thoma NH (2013) Rif1 and Rif2 shape telomere function and architecture through multivalent Rap1 interactions. Cell 153 (6):1340-1353. doi:10.1016/j.cell.2013.05.007
50. Mattarocci S, Hafner L, Lezaja A, Shyian M, Shore D (2016) Rif1: A Conserved Regulator of DNA Replication and Repair Hijacked by Telomeres in Yeasts. Frontiers in genetics 7:45. doi:10.3389/fgene.2016.00045
51. Buonomo SBC (2017) Rif1-Dependent Regulation of Genome Replication in Mammals. Adv Exp Med Biol 1042:259-272. doi:10.1007/978-981-10-6955-0_12
52. Silverman J, Takai H, Buonomo SB, Eisenhaber F, de Lange T (2004) Human Rif1, ortholog of a yeast telomeric protein, is regulated by ATM and 53BP1 and functions in the S-phase checkpoint. Genes Dev 18 (17):2108-2119. doi:10.1101/gad.1216004
18/17/2108 [pii]
53. Yang S, Liu L, Cao C, Song N, Wang Y, Ma S, Zhang Q, Yu N, Ding X, Yang F, Tian S, Zhang K, Sun T, Yang J, Yao Z, Wu S, Shi L (2018) USP52 acts as a deubiquitinase and promotes histone chaperone ASF1A stabilization. Nature communications 9 (1):1285. doi:10.1038/s41467-018-03588-z
54. Sukackaite R, Cornacchia D, Jensen MR, Mas PJ, Blackledge M, Enervald E, Duan G, Auchynnikava T, Kohn M, Hart DJ, Buonomo SBC (2017) Mouse Rif1 is a regulatory subunit of protein phosphatase 1 (PP1). Scientific reports 7 (1):2119. doi:10.1038/s41598-017-01910-1
55. Srivastava M, Chen Z, Zhang H, Tang M, Wang C, Jung SY, Chen J (2018) Replisome Dynamics and Their Functional Relevance upon DNA Damage through the PCNA Interactome. Cell Rep 25 (13):3869-3883 e3864. doi:10.1016/j.celrep.2018.11.099
56. Gupta R, Somyajit K, Narita T, Maskey E, Stanlie A, Kremer M, Typas D, Lammers M, Mailand N, Nussenzweig A, Lukas J, Choudhary C (2018) DNA Repair Network Analysis

Reveals Shieldin as a Key Regulator of NHEJ and PARP Inhibitor Sensitivity. Cell 173 (4):972-988 e923. doi:10.1016/j.cell.2018.03.050
57. Lee KY, Im JS, Shibata E, Dutta A (2017) ASF1a Promotes Non-homologous End Joining Repair by Facilitating Phosphorylation of MDC1 by ATM at Double-Strand Breaks. Mol Cell 68 (1):61-75 e65. doi:10.1016/j.molcel.2017.08.021
58. Roe JL, Durfee T, Zupan JR, Repetti PP, McLean BG, Zambryski PC (1997) TOUSLED is a nuclear serine/threonine protein kinase that requires a coiled-coil region for oligomerization and catalytic activity. J Biol Chem 272 (9):5838-5845
59. Hartford SA, Luo Y, Southard TL, Min IM, Lis JT, Schimenti JC (2011) Minichromosome maintenance helicase paralog MCM9 is dispensible for DNA replication but functions in germ-line stem cells and tumor suppression. Proc Natl Acad Sci U S A 108 (43):17702-17707. doi:10.1073/pnas.1113524108
60. Lai M, Liang L, Chen J, Qiu N, Ge S, Ji S, Shi T, Zhen B, Liu M, Ding C, Wang Y, Qin J (2016) Multidimensional Proteomics Reveals a Role of UHRF2 in the Regulation of Epithelial-Mesenchymal Transition (EMT). Mol Cell Proteomics 15 (7):2263-2278. doi:10.1074/mcp.M115.057448
61. Wang J, Huo K, Ma L, Tang L, Li D, Huang X, Yuan Y, Li C, Wang W, Guan W, Chen H, Jin C, Wei J, Zhang W, Yang Y, Liu Q, Zhou Y, Zhang C, Wu Z, Xu W, Zhang Y, Liu T, Yu D, Zhang Y, Chen L, Zhu D, Zhong X, Kang L, Gan X, Yu X, Ma Q, Yan J, Zhou L, Liu Z, Zhu Y, Zhou T, He F, Yang X (2011) Toward an understanding of the protein interaction network of the human liver. Mol Syst Biol 7:536. doi:10.1038/msb.2011.67
62. Correia SP, Chan AB, Vaughan M, Zolboot N, Perea V, Huber AL, Kriebs A, Moresco JJ, Yates JR, 3rd, Lamia KA (2019) The circadian E3 ligase complex SCF(FBXL3+CRY) targets TLK2. Scientific reports 9 (1):198. doi:10.1038/s41598-018-36618-3
63. Liu H, Dowdle JA, Khurshid S, Sullivan NJ, Bertos N, Rambani K, Mair M, Daniel P, Wheeler E, Tang X, Toth K, Lause M, Harrigan ME, Eiring K, Sullivan C, Sullivan MJ, Chang SW, Srivastava S, Conway JS, Kladney R, McElroy J, Bae S, Lu Y, Tofigh A, Saleh SMI, Fernandez SA, Parvin JD, Coppola V, Macrae ER, Majumder S, Shapiro CL, Yee LD, Ramaswamy B, Hallett M, Ostrowski MC, Park M, Chamberlin HM, Leone G (2017) Discovery of Stromal Regulatory Networks that Suppress Ras-Sensitized Epithelial Cell Proliferation. Dev Cell 41 (4):392-407 e396. doi:10.1016/j.devcel.2017.04.024
64. Emili A, Schieltz DM, Yates JR, 3rd, Hartwell LH (2001) Dynamic interaction of DNA damage checkpoint protein Rad53 with chromatin assembly factor Asf1. Mol Cell 7 (1):13-20
65. Hu F, Alcasabas AA, Elledge SJ (2001) Asf1 links Rad53 to control of chromatin assembly. Genes Dev 15 (9):1061-1066. doi:10.1101/gad.873201
66. Blasius M, Forment JV, Thakkar N, Wagner SA, Choudhary C, Jackson SP (2011) A phospho-proteomic screen identifies substrates of the checkpoint kinase Chk1. Genome Biol 12 (8):R78. doi:10.1186/gb-2011-12-8-r78
67. Bruinsma W, van den Berg J, Aprelia M, Medema RH (2016) Tousled-like kinase 2 regulates recovery from a DNA damage-induced G2 arrest. EMBO Rep 17 (5):659-670. doi:10.15252/embr.201540767
68. Kim JA, Anurag M, Veeraraghavan J, Schiff R, Li K, Wang X (2016) Amplification of TLK2 Induces Genomic Instability via Impairing the G2/M Checkpoint. Mol Cancer Res. doi:10.1158/1541-7786.MCR-16-0161
69. Liaw GJ, Chiang CS (2019) Inactive Tlk associating with Tak1 increases p38 MAPK activity to prolong the G2 phase. Scientific reports 9 (1):1885. doi:10.1038/s41598-018-36137-1

70. Sunavala-Dossabhoy G, Li Y, Williams B, De Benedetti A (2003) A dominant negative mutant of TLK1 causes chromosome missegregation and aneuploidy in normal breast epithelial cells. BMC Cell Biol 4:16. doi:10.1186/1471-2121-4-16
1471-2121-4-16 [pii]
71. Hashimoto M, Matsui T, Iwabuchi K, Date T (2008) PKU-beta/TLK1 regulates myosin II activities, and is required for accurate equaled chromosome segregation. Mutat Res 657 (1):63-67. doi:S1383-5718(08)00262-3 [pii]
10.1016/j.mrgentox.2008.09.001
72. Gaillard H, Garcia-Muse T, Aguilera A (2015) Replication stress and cancer. Nat Rev Cancer 15 (5):276-289. doi:10.1038/nrc3916
73. Saredi G, Huang H, Hammond CM, Alabert C, Bekker-Jensen S, Forne I, Reveron-Gomez N, Foster BM, Mlejnkova L, Bartke T, Cejka P, Mailand N, Imhof A, Patel DJ, Groth A (2016) H4K20me0 marks post-replicative chromatin and recruits the TONSL-MMS22L DNA repair complex. Nature 534 (7609):714-718. doi:10.1038/nature18312
74. Toledo LI, Altmeyer M, Rask MB, Lukas C, Larsen DH, Povlsen LK, Bekker-Jensen S, Mailand N, Bartek J, Lukas J (2013) ATR prohibits replication catastrophe by preventing global exhaustion of RPA. Cell 155 (5):1088-1103. doi:10.1016/j.cell.2013.10.043
75. Chapman JR, Taylor MR, Boulton SJ (2012) Playing the end game: DNA double-strand break repair pathway choice. Mol Cell 47 (4):497-510. doi:S1097-2765(12)00656-9 [pii]
10.1016/j.molcel.2012.07.029
76. Groth A, Corpet A, Cook AJ, Roche D, Bartek J, Lukas J, Almouzni G (2007) Regulation of replication fork progression through histone supply and demand. Science 318 (5858):1928-1931. doi:318/5858/1928 [pii]
10.1126/science.1148992
77. Huang H, Stromme CB, Saredi G, Hodl M, Strandsby A, Gonzalez-Aguilera C, Chen S, Groth A, Patel DJ (2015) A unique binding mode enables MCM2 to chaperone histones H3-H4 at replication forks. Nat Struct Mol Biol 22 (8):618-626. doi:10.1038/nsmb.3055
78. Dillon PJ, Gregory SM, Tamburro K, Sanders MK, Johnson GL, Raab-Traub N, Dittmer DP, Damania B (2013) Tousled-like Kinases Modulate Reactivation of Gammaherpesviruses from Latency. Cell Host Microbe 13 (2):204-214. doi:S1931-3128(13)00033-4 [pii]
10.1016/j.chom.2012.12.005
79. O'Sullivan RJ, Arnoult N, Lackner DH, Oganesian L, Haggblom C, Corpet A, Almouzni G, Karlseder J (2014) Rapid induction of alternative lengthening of telomeres by depletion of the histone chaperone ASF1. Nat Struct Mol Biol 21 (2):167-174. doi:10.1038/nsmb.2754
80. Houlard M, Berlivet S, Probst AV, Quivy JP, Hery P, Almouzni G, Gerard M (2006) CAF-1 is essential for heterochromatin organization in pluripotent embryonic cells. PLoS Genet 2 (11):e181. doi:06-PLGE-RA-0236R2 [pii]
10.1371/journal.pgen.0020181
81. Udugama M, FT MC, Chan FL, Tang MC, Pickett HA, JD RM, Mayne L, Collas P, Mann JR, Wong LH (2015) Histone variant H3.3 provides the heterochromatic H3 lysine 9 tri-methylation mark at telomeres. Nucleic Acids Res 43 (21):10227-10237. doi:10.1093/nar/gkv847
82. Elsasser SJ, Noh KM, Diaz N, Allis CD, Banaszynski LA (2015) Histone H3.3 is required for endogenous retroviral element silencing in embryonic stem cells. Nature 522 (7555):240-244. doi:10.1038/nature14345
83. Banaszynski LA, Wen D, Dewell S, Whitcomb SJ, Lin M, Diaz N, Elsasser SJ, Chapgier A, Goldberg AD, Canaani E, Rafii S, Zheng D, Allis CD (2013) Hira-dependent histone H3.3 deposition facilitates PRC2 recruitment at developmental loci in ES cells. Cell 155 (1):107-120. doi:10.1016/j.cell.2013.08.061

84. Segura-Bayona S, Villamor-Paya M, Attolini CS, Stracker TH (2019) Tousled-like kinase activity is required for transcriptional silencing and suppression of innate immune signaling. bioRxiv. doi:https://doi.org/10.1101/621409
85. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C, Schultz N (2013) Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 6 (269):pl1. doi:10.1126/scisignal.2004088
86. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C, Schultz N (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2 (5):401-404. doi:10.1158/2159-8290.CD-12-0095
87. Lecona E, Fernandez-Capetillo O (2018) Targeting ATR in cancer. Nat Rev Cancer 18 (9):586-595. doi:10.1038/s41568-018-0034-3
88. Lelieveld SH, Reijnders MR, Pfundt R, Yntema HG, Kamsteeg EJ, de Vries P, de Vries BB, Willemsen MH, Kleefstra T, Lohner K, Vreeburg M, Stevens SJ, van der Burgt I, Bongers EM, Stegmann AP, Rump P, Rinne T, Nelen MR, Veltman JA, Vissers LE, Brunner HG, Gilissen C (2016) Meta-analysis of 2,104 trios provides support for 10 new genes for intellectual disability. Nature neuroscience 19 (9):1194-1196. doi:10.1038/nn.4352
89. Reijnders MRF, Miller KA, Alvi M, Goos JAC, Lees MM, de Burca A, Henderson A, Kraus A, Mikat B, de Vries BBA, Isidor B, Kerr B, Marcelis C, Schluth-Bolard C, Deshpande C, Ruivenkamp CAL, Wieczorek D, Deciphering Developmental Disorders S, Baralle D, Blair EM, Engels H, Ludecke HJ, Eason J, Santen GWE, Clayton-Smith J, Chandler K, Tatton-Brown K, Payne K, Helbig K, Radtke K, Nugent KM, Cremer K, Strom TM, Bird LM, Sinnema M, Bitner-Glindzicz M, van Dooren MF, Alders M, Koopmans M, Brick L, Kozenko M, Harline ML, Klaassens M, Steinraths M, Cooper NS, Edery P, Yap P, Terhal PA, van der Spek PJ, Lakeman P, Taylor RL, Littlejohn RO, Pfundt R, Mercimek-Andrews S, Stegmann APA, Kant SG, McLean S, Joss S, Swagemakers SMA, Douzgou S, Wall SA, Kury S, Calpena E, Koelling N, McGowan SJ, Twigg SRF, Mathijssen IMJ, Nellaker C, Brunner HG, Wilkie AOM (2018) De Novo and Inherited Loss-of-Function Variants in TLK2: Clinical and Genotype-Phenotype Evaluation of a Distinct Neurodevelopmental Disorder. Am J Hum Genet 102 (6):1195-1203. doi:10.1016/j.ajhg.2018.04.014
90. Takata A, Miyake N, Tsurusaki Y, Fukai R, Miyatake S, Koshimizu E, Kushima I, Okada T, Morikawa M, Uno Y, Ishizuka K, Nakamura K, Tsujii M, Yoshikawa T, Toyota T, Okamoto N, Hiraki Y, Hashimoto R, Yasuda Y, Saitoh S, Ohashi K, Sakai Y, Ohga S, Hara T, Kato M, Nakamura K, Ito A, Seiwa C, Shirahata E, Osaka H, Matsumoto A, Takeshita S, Tohyama J, Saikusa T, Matsuishi T, Nakamura T, Tsuboi T, Kato T, Suzuki T, Saitsu H, Nakashima M, Mizuguchi T, Tanaka F, Mori N, Ozaki N, Matsumoto N (2018) Integrative Analyses of De Novo Mutations Provide Deeper Biological Insights into Autism Spectrum Disorder. Cell Rep 22 (3):734-747. doi:10.1016/j.celrep.2017.12.074
91. Kelemen LE, Wang X, Fredericksen ZS, Pankratz VS, Pharoah PD, Ahmed S, Dunning AM, Easton DF, Vierkant RA, Cerhan JR, Goode EL, Olson JE, Couch FJ (2009) Genetic variation in the chromosome 17q23 amplicon and breast cancer risk. Cancer Epidemiol Biomarkers Prev 18 (6):1864-1868. doi:10.1158/1055-9965.EPI-08-0486
92. Stevens KN, Wang X, Fredericksen Z, Pankratz VS, Cerhan J, Vachon CM, Olson JE, Couch FJ (2011) Evaluation of associations between common variation in mitotic regulatory pathways and risk of overall and high grade breast cancer. Breast Cancer Res Treat 129 (2):617-622. doi:10.1007/s10549-011-1587-y
93. Kim JA, Tan Y, Wang X, Cao X, Veeraraghavan J, Liang Y, Edwards DP, Huang S, Pan X, Li K, Schiff R, Wang XS (2016) Comprehensive functional analysis of the tousled-like

kinase 2 frequently amplified in aggressive luminal breast cancers. Nature communications 7:12991. doi:10.1038/ncomms12991
94. Mertins P, Mani DR, Ruggles KV, Gillette MA, Clauser KR, Wang P, Wang X, Qiao JW, Cao S, Petralia F, Kawaler E, Mundt F, Krug K, Tu Z, Lei JT, Gatza ML, Wilkerson M, Perou CM, Yellapantula V, Huang KL, Lin C, McLellan MD, Yan P, Davies SR, Townsend RR, Skates SJ, Wang J, Zhang B, Kinsinger CR, Mesri M, Rodriguez H, Ding L, Paulovich AG, Fenyo D, Ellis MJ, Carr SA (2016) Proteogenomics connects somatic mutations to signalling in breast cancer. Nature 534 (7605):55-62. doi:10.1038/nature18003
95. Singh V, Jaiswal PK, Ghosh I, Koul HK, Yu X, De Benedetti A (2019) The TLK1-Nek1 axis promotes prostate cancer progression. Cancer Lett 453:131-141. doi:10.1016/j.canlet.2019.03.041
96. Singh V, Jaiswal PK, Ghosh I, Koul HK, Yu X, De Benedetti A (2019) Targeting the TLK1/NEK1 DDR axis with Thioridazine suppresses outgrowth of androgen independent prostate tumors. Int J Cancer. doi:10.1002/ijc.32200
97. Takayama Y, Kokuryo T, Yokoyama Y, Ito S, Nagino M, Hamaguchi M, Senga T (2010) Silencing of Tousled-like kinase 1 sensitizes cholangiocarcinoma cells to cisplatin-induced apoptosis. Cancer Lett 296 (1):27-34. doi:S0304-3835(10)00148-5 [pii]
10.1016/j.canlet.2010.03.011
98. Hu S, Wang H, Yan D, Lu W, Gao P, Lou W, Kong X (2018) Loss of miR-16 contributes to tumor progression by activation of tousled-like kinase 1 in oral squamous cell carcinoma. Cell Cycle 17 (18):2284-2295. doi:10.1080/15384101.2018.1526601
99. Ronald S, Awate S, Rath A, Carroll J, Galiano F, Dwyer D, Kleiner-Hancock H, Mathis JM, Vigod S, De Benedetti A (2013) Phenothiazine Inhibitors of TLKs Affect Double-Strand Break Repair and DNA Damage Response Recovery and Potentiate Tumor Killing with Radiomimetic Therapy. Genes Cancer 4 (1-2):39-53. doi:10.1177/1947601913479020
100. O'Roak BJ, Deriziotis P, Lee C, Vives L, Schwartz JJ, Girirajan S, Karakoc E, Mackenzie AP, Ng SB, Baker C, Rieder MJ, Nickerson DA, Bernier R, Fisher SE, Shendure J, Eichler EE (2011) Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. Nat Genet 43 (6):585-589. doi:10.1038/ng.835
101. Gulsuner S, Walsh T, Watts AC, Lee MK, Thornton AM, Casadei S, Rippey C, Shahin H, Consortium on the Genetics of S, Group PS, Nimgaonkar VL, Go RC, Savage RM, Swerdlow NR, Gur RE, Braff DL, King MC, McClellan JM (2013) Spatial and temporal mapping of de novo mutations in schizophrenia to a fetal prefrontal cortical network. Cell 154 (3):518-529. doi:10.1016/j.cell.2013.06.049
102. Fromer M, Pocklington AJ, Kavanagh DH, Williams HJ, Dwyer S, Gormley P, Georgieva L, Rees E, Palta P, Ruderfer DM, Carrera N, Humphreys I, Johnson JS, Roussos P, Barker DD, Banks E, Milanova V, Grant SG, Hannon E, Rose SA, Chambert K, Mahajan M, Scolnick EM, Moran JL, Kirov G, Palotie A, McCarroll SA, Holmans P, Sklar P, Owen MJ, Purcell SM, O'Donovan MC (2014) De novo mutations in schizophrenia implicate synaptic networks. Nature 506 (7487):179-184. doi:10.1038/nature12929
103. De Rubeis S, He X, Goldberg AP, Poultney CS, Samocha K, Cicek AE, Kou Y, Liu L, Fromer M, Walker S, Singh T, Klei L, Kosmicki J, Shih-Chen F, Aleksic B, Biscaldi M, Bolton PF, Brownfeld JM, Cai J, Campbell NG, Carracedo A, Chahrour MH, Chiocchetti AG, Coon H, Crawford EL, Curran SR, Dawson G, Duketis E, Fernandez BA, Gallagher L, Geller E, Guter SJ, Hill RS, Ionita-Laza J, Jimenz Gonzalez P, Kilpinen H, Klauck SM, Kolevzon A, Lee I, Lei I, Lei J, Lehtimaki T, Lin CF, Ma'ayan A, Marshall CR, McInnes AL, Neale B, Owen MJ, Ozaki N, Parellada M, Parr JR, Purcell S, Puura K, Rajagopalan D, Rehnstrom K, Reichenberg A, Sabo A, Sachse M, Sanders SJ, Schafer C, Schulte-Ruther M, Skuse D, Stevens C, Szatmari P, Tammimies K, Valladares O, Voran A, Li-San W, Weiss LA, Willsey AJ, Yu TW, Yuen RK, Study DDD, Homozygosity Mapping Collaborative for

A, Consortium UK, Cook EH, Freitag CM, Gill M, Hultman CM, Lehner T, Palotie A, Schellenberg GD, Sklar P, State MW, Sutcliffe JS, Walsh CA, Scherer SW, Zwick ME, Barett JC, Cutler DJ, Roeder K, Devlin B, Daly MJ, Buxbaum JD (2014) Synaptic, transcriptional and chromatin genes disrupted in autism. Nature 515 (7526):209-215. doi:10.1038/nature13772
104. Homsy J, Zaidi S, Shen Y, Ware JS, Samocha KE, Karczewski KJ, DePalma SR, McKean D, Wakimoto H, Gorham J, Jin SC, Deanfield J, Giardini A, Porter GA, Jr., Kim R, Bilguvar K, Lopez-Giraldez F, Tikhonova I, Mane S, Romano-Adesman A, Qi H, Vardarajan B, Ma L, Daly M, Roberts AE, Russell MW, Mital S, Newburger JW, Gaynor JW, Breitbart RE, Iossifov I, Ronemus M, Sanders SJ, Kaltman JR, Seidman JG, Brueckner M, Gelb BD, Goldmuntz E, Lifton RP, Seidman CE, Chung WK (2015) De novo mutations in congenital heart disease with neurodevelopmental and other congenital anomalies. Science 350 (6265):1262-1266. doi:10.1126/science.aac9396
105. McKinnon PJ (2017) Genome integrity and disease prevention in the nervous system. Genes Dev 31 (12):1180-1194. doi:10.1101/gad.301325.117
106. Murga M, Bunting S, Montana MF, Soria R, Mulero F, Canamero M, Lee Y, McKinnon PJ, Nussenzweig A, Fernandez-Capetillo O (2009) A mouse model of ATR-Seckel shows embryonic replicative stress and accelerated aging. Nat Genet 41 (8):891-898. doi:ng.420 [pii]
10.1038/ng.420
107. McNairn AJ, Chuang CH, Bloom JC, Wallace MD, Schimenti JC (2019) Female-biased embryonic death from inflammation induced by genomic instability. Nature 567 (7746):105-108. doi:10.1038/s41586-019-0936-6
108. Lee J, Kim MS, Park SH, Jang YK (2018) Tousled-like kinase 1 is a negative regulator of core transcription factors in murine embryonic stem cells. Scientific reports 8 (1):334. doi:10.1038/s41598-017-18628-9
109. Sadic D, Schmidt K, Groh S, Kondofersky I, Ellwart J, Fuchs C, Theis FJ, Schotta G (2015) Atrx promotes heterochromatin formation at retrotransposons. EMBO Rep 16 (7):836-850. doi:10.15252/embr.201439937
110. Abrahams BS, Arking DE, Campbell DB, Mefford HC, Morrow EM, Weiss LA, Menashe I, Wadkins T, Banerjee-Basu S, Packer A (2013) SFARI Gene 2.0: a community-driven knowledgebase for the autism spectrum disorders (ASDs). Mol Autism 4 (1):36. doi:10.1186/2040-2392-4-36
111. Cipriani C, Ricceri L, Matteucci C, De Felice A, Tartaglione AM, Argaw-Denboba A, Pica F, Grelli S, Calamandrei G, Sinibaldi Vallebona P, Balestrieri E (2018) High expression of Endogenous Retroviruses from intrauterine life to adulthood in two mouse models of Autism Spectrum Disorders. Scientific reports 8 (1):629. doi:10.1038/s41598-017-19035-w
112. Christensen J, Gronborg TK, Sorensen MJ, Schendel D, Parner ET, Pedersen LH, Vestergaard M (2013) Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. JAMA 309 (16):1696-1703. doi:10.1001/jama.2013.2270
113. Irimia M, Weatheritt RJ, Ellis JD, Parikshak NN, Gonatopoulos-Pournatzis T, Babor M, Quesnel-Vallieres M, Tapial J, Raj B, O'Hanlon D, Barrios-Rodiles M, Sternberg MJ, Cordes SP, Roth FP, Wrana JL, Geschwind DH, Blencowe BJ (2014) A highly conserved program of neuronal microexons is misregulated in autistic brains. Cell 159 (7):1511-1523. doi:10.1016/j.cell.2014.11.035
114. Quesnel-Vallieres M, Dargaei Z, Irimia M, Gonatopoulos-Pournatzis T, Ip JY, Wu M, Sterne-Weiler T, Nakagawa S, Woodin MA, Blencowe BJ, Cordes SP (2016) Misregulation of an Activity-Dependent Splicing Network as a Common Mechanism Underlying Autism Spectrum Disorders. Mol Cell 64 (6):1023-1034. doi:10.1016/j.molcel.2016.11.033

115. Gonatopoulos-Pournatzis T, Wu M, Braunschweig U, Roth J, Han H, Best AJ, Raj B, Aregger M, O'Hanlon D, Ellis JD, Calarco JA, Moffat J, Gingras AC, Blencowe BJ (2018) Genome-wide CRISPR-Cas9 Interrogation of Splicing Networks Reveals a Mechanism for Recognition of Autism-Misregulated Neuronal Microexons. Mol Cell 72 (3):510-524 e512. doi:10.1016/j.molcel.2018.10.008
116. Guo R, Zheng L, Park JW, Lv R, Chen H, Jiao F, Xu W, Mu S, Wen H, Qiu J, Wang Z, Yang P, Wu F, Hui J, Fu X, Shi X, Shi YG, Xing Y, Lan F, Shi Y (2014) BS69/ZMYND11 reads and connects histone H3.3 lysine 36 trimethylation-decorated chromatin to regulated pre-mRNA processing. Mol Cell 56 (2):298-310. doi:10.1016/j.molcel.2014.08.022
117. Jimeno-Gonzalez S, Payan-Bravo L, Munoz-Cabello AM, Guijo M, Gutierrez G, Prado F, Reyes JC (2015) Defective histone supply causes changes in RNA polymerase II elongation rate and cotranscriptional pre-mRNA splicing. Proc Natl Acad Sci U S A 112 (48):14840-14845. doi:10.1073/pnas.1506760112
118. Xie J, Huang L, Li X, Li H, Zhou Y, Zhu H, Pan T, Kendrick KM, Xu W (2017) Immunological cytokine profiling identifies TNF-alpha as a key molecule dysregulated in autistic children. Oncotarget 8 (47):82390-82398. doi:10.18632/oncotarget.19326
119. Choi GB, Yim YS, Wong H, Kim S, Kim H, Kim SV, Hoeffer CA, Littman DR, Huh JR (2016) The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. Science 351 (6276):933-939. doi:10.1126/science.aad0314
120. Kim S, Kim H, Yim YS, Ha S, Atarashi K, Tan TG, Longman RS, Honda K, Littman DR, Choi GB, Huh JR (2017) Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. Nature 549 (7673):528-532. doi:10.1038/nature23910
121. Murga M, Campaner S, Lopez-Contreras AJ, Toledo LI, Soria R, Montana MF, D'Artista L, Schleker T, Guerra C, Garcia E, Barbacid M, Hidalgo M, Amati B, Fernandez-Capetillo O (2011) Exploiting oncogene-induced replicative stress for the selective killing of Myc-driven tumors. Nat Struct Mol Biol 18 (12):1331-1335. doi:nsmb.2189 [pii]
10.1038/nsmb.2189
122. Carpentier PA, Dingman AL, Palmer TD (2011) Placental TNF-alpha signaling in illness-induced complications of pregnancy. Am J Pathol 178 (6):2802-2810. doi:10.1016/j.ajpath.2011.02.042